Antiischemic Properties of Fasudil in Experimental Models of Vasospastic Angina

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ABSTRACT—We studied the antiischemic properties of fasudil, a Rho-kinase inhibitor, in conscious rabbits with coronary vasospasm induced by vasopressin and endothelin. Pretreatment with fasudil (0.3 and 3 mg/kg) attenuated the maximum elevation of the T-wave elicited by endothelin. Pretreatment with fasudil inhibited the T-wave elevation elicited by vasopressin. Fasudil and hydroxy fasudil, an active metabolite of fasudil, relaxed the endothelin-, U-46619-, 5-hydroxytryptamine- or histamine-induced contraction in swine coronary arterial strips. Fasudil and hydroxy fasudil significantly prevented the reduction in coronary flow by vasopressin in the Langendorff perfused rat heart. Fasudil was effective in protecting the heart against vasopressin and endothelin-induced myocardial ischemic change in conscious rabbits, and this beneficial effect can be attributed to its action of ameliorating the severe contraction of arteries. The inhibition of Rho-kinase may have implications for the development of novel therapeutic strategies for vasospastic angina in patients.

Keywords: Coronary vasospasm, Angina, Rho-kinase inhibitor, Fasudil, Hydroxy fasudil

Fasudil is a protein kinase inhibitor that inhibits Rho-kinase more effectively than it inhibits other protein kinases such as protein kinase C (PKC) and myosin light chain kinase (MLCK) (1–3). Rho-kinase is shown to be capable of phosphorylating the myosin binding subunit (MBS) of myosin phosphatase and inhibiting myosin phosphatase activity and enhances myosin light chain (MLC) phosphorylation and contraction in vascular smooth muscle (3). Fasudil inhibits the phosphorylation of MBS and MLC and relaxes the vascular contractions. In a clinical trial and experimental studies, fasudil was metabolized to the active metabolite hydroxy fasudil (4, 5), which also preferentially inhibits Rho-kinase (6).

To our knowledge, fasudil was the first drug that angiographically dilated the spastic basilar artery during subarachnoid hemorrhage on day 7 via intravenous administration of doses that caused the systemic blood pressure to fall only slightly in the two-hemorrhage canine model (7). In clinical trials, fasudil reduced angiographically demonstrable cerebral vasospasm and reduced the number of patients with a poor clinical outcome associated with cerebral vasospasm (4). Fasudil was launched for clinical use after subarachnoid hemorrhage in Japan.

Previous findings suggested that fasudil, which is a potent vasodilator whose mechanism of vasodilator action differs from currently available antianginal drugs, may also have beneficial effects in coronary vasospasm. Katsumata et al. (8) reported that fasudil inhibited the contractions and MLC phosphorylation of the spastic coronary arteries in a swine model with interleukin-1β.

We studied whether fasudil protected the heart from ischemia in two animal models of vasospastic angina. The T-wave elevation in the electrocardiogram (ECG) was used as the index of severity of myocardial ischemia and function, because it has been reported that T-wave elevation is a direct effect of cardiac anoxia and injuries subsequent to coronary vasoconstriction (9). These studies were conducted in conscious animals to eliminate the potentially confounding influence of factors such as anesthesia.

MATERIALS AND METHODS

In vivo studies

All animals were used in accordance with ethical procedures approved for the care and use of laboratory animals
Antiischemic Properties of Fasudil

by The Japanese Pharmacological Society. The method of inducing coronary vasospasm by vasopressin or endothelin in rabbits was essentially the same as in the previous studies (9, 10). Male Japanese white rabbits, weighing 2.2 – 3.3 kg, were anesthetized with pentobarbital sodium (35 mg/kg, i.v.). ECG pin electrodes were implanted subcutaneously in the right and left shoulders and flanks. The rabbits were used following a post-surgery recovery time of at least 4 days. The rabbits were fasted for 24 h, and vehicle or drugs were administered orally. Fifteen minutes after the administration of drugs, lysine-vasopressin (0.05 IU/kg) or endothelin (1.4 nmol/kg) was injected intravenously. The lead II of ECG was recorded before the administration of drugs; before the administration of vasopressin or endothelin; and at 3, 5, 10, 15 and 30 min after the administration of vasopressin or endothelin. The magnitude of T-wave elevation was analyzed using an analyzing system, which consisted of a personal computer (PC-9801EX; NEC, Tokyo) and software (GENIUS ECG; MRE, Tokyo).

**In vitro studies**

The porcine hearts were obtained from a local abattoir. The coronary arteries were dissected and cut into rings. The arterial rings were mounted isometrically under 5 g of tension in a 20-ml tissue bath (37°C) containing Krebs-Henseleit solution and aerated with a mixture of 95% O₂ and 5% CO₂. Force generation was monitored with an isometric transducer. KCl solution (62 mM) was applied until the amplitude of the contraction reached a constant value. The application of endothelin (30 nM); U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F₂α), a synthetic agonist of the thromboxane receptor, (10 nM); 5-hydroxytryptamine (5-HT, 1 µM); or histamine (3 µM) resulted in stable contractions. Fasudil or hydroxy fasudil was added cumulatively to the tissue bath to obtain a concentration-response curve.

Male Wistar rats (220 – 260 g) were administered heparin (500 U/kg, i.p.) 15 min before the heart was excised. Isolated hearts were perfused by an aortic cannula with Krebs-Henseleit buffer according to the method of Langendorff (11, 12). Coronary flow was measured using an electromagnetic flow probe of the extracorporeal type. The hearts were beating spontaneously throughout the experimental procedure. After the stabilization period, hearts were perfused with the Krebs-Henseleit solution containing either fasudil or hydroxy fasudil. When the increases in coronary flow had reached a plateau, vasopressin (3 mM) was added.

**Statistical analyses**

The values are expressed as the mean ± S.E.M. The significance of difference was calculated by Dunnett’s test. *P* values of 0.05 or less were considered to indicate significant differences.

**Drugs and chemicals**

The drugs used were fasudil (Asahi Kasei Corporation, Tokyo); nicorandil (Chugai, Tokyo); nifedipine (Bayer, Leverkusen, Germany); endothelin (Peptide Institute Inc., Osaka); vasopressin, U-46619, 5-HT and histamine (Sigma Chemical Co., St. Louis, MO, USA).

**RESULTS**

**In vivo studies**

Intravenous bolus injection of vasopressin (0.05 IU/kg) or endothelin (1.4 nmol/kg) into conscious rabbits induced T-wave elevation in the ECG (Fig. 1). The maximum elevation of the T-wave by endothelin or vasopressin was observed 5 or 3 min after administration, respectively (Figs. 2 and 3).

Pretreatment of the animals with fasudil (0.3 and 3 mg/kg, p.o.) attenuated the maximum elevation of the T-wave elicited by endothelin in a dose-dependent manner (Fig. 2).

![Fig. 1. Typical tracings showing the inhibitory effect of fasudil on the T-wave elevation caused by endothelin in rabbits. Endothelin (1.4 nmol/kg) was administered intravenously at time 0. Fasudil (3 mg/kg) was administered orally 15 min before (-15 min) the administration of endothelin.](image-url)
Nicorandil (3 mg/kg, p.o.) and nifedipine (3 mg/kg, p.o.) also attenuated the T-wave elevation, although diltiazem (0.3 and 3 mg/kg, p.o.) failed to attenuate the maximum elevation of the T-wave elicited by endothelin 5 min after the administration of endothelin (Fig. 2). Diltiazem (0.3 and 3 mg/kg, p.o.) attenuated the T-wave elevation only at 10 min after the administration of endothelin.

The increase in the heart rate produced by 3 mg/kg of fasudil, nicorandil, diltiazem or nifedipine was significant when compared with the corresponding value for the control (group with endothelin-treatment alone, Table 1). When 0.3 mg/kg of fasudil, nicorandil or diltiazem was administered, there was no significant increase in the heart rate (data not shown). Nifedipine (0.3 mg/kg) significantly increased heart rate (data not shown). The heart rate measured before and after endothelin administration remained unchanged (Table 1).

Pretreatment of the animals with fasudil (3 mg/kg, p.o.) or diltiazem (3 mg/kg, p.o.) inhibited the maximum elevation of the T-wave elicited by vasopressin by an average of 58% or 53%, respectively (Fig. 3). In contrast, no significant inhibition was observed with nicorandil (0.3 and 3 mg/kg, p.o.) (data not shown).

In vitro studies

In swine coronary arterial strips contracted by endothelin, U-46619, 5-HT or histamine, the fasudil or hydroxy fasudil-induced relaxation was concentration-dependent, and it was similarly potent in inhibiting contraction induced by all the agonists tested (Fig. 4). The magnitude of the vasodilatory responses of hydroxy fasudil was similar to those of fasudil.

Administration of vasopressin as a bolus reduced the coronary flow dose-dependently in the Langendorff perfused rat heart (Fig. 5). Fasudil and hydroxy fasudil (3 μM) significantly prevented the reduction in the coronary flow by vasopressin (3 mM) (Fig. 6).
DISCUSSION

The intensity of T-wave elevation in the ECG has been found to be a useful index of myocardial ischemia caused by coronary vasoconstriction (9). The present findings showed that i.v. injection of vasopressin or endothelin produced T-wave elevation in awake rabbits. These findings are consistent with the previous observations that intravenous administration of vasopressin or endothelin causes a rise in T-wave (9, 10).

Endothelin has recently been recognized as one of the key mediators of myocardial ischemia (13). Elevated plasma concentrations of endothelin can be detected in patients exhibiting coronary spasm (14). Endothelin is a potent constrictor of coronary arteries (15). Furthermore, administration of endothelin evokes ECG changes (10, 16), similar to the clinical phenomenon of angina, especially variant angina. Yamamoto et al. (10) reported that endothelin induced myocardial dysfunction in rabbits and fasudil prevented the occurrence of endothelin-induced...
ischemic myocardial injury. In the present study, intravenous injection of endothelin also induced T-wave elevation; and fasudil, nicorandil, nifedipine and diltiazem inhibited the T-wave elevation after the administration of endothelin. A low dose (0.3 mg/kg) of fasudil attenuated the maximum elevation of the T-wave after the administration of endothelin, although a low dose (0.3 mg/kg) of nicardipine, nifedipine or diltiazem failed to attenuate the maximum elevation of the T-wave. Fasudil was equally or more potent than antianginal drugs used in the present study to ameliorate the coronary vasospasm induced by vasopressin.

In an earlier preliminary trial, intraduodenal administration of fasudil (0.1 – 1 mg/kg) had no effect on the blood pressure in anesthetized rabbits (unpublished observations). It is considered that at least 0.3 mg/kg fasudil does not affect the blood pressure; however, in the present studies, we did not measure the effect of fasudil on blood pressure, heart rate or myocardial contractility in the models of vasospastic angina or in the Langendorff perfused rat heart. To define the mechanisms of fasudil in protecting the heart from ischemia, it may be useful to examine the effect of fasudil on blood pressure, heart rate or myocardial contractility. It may also be useful to examine the effect of fasudil on the change in the coronary flow caused by endo-
endothelin in the Langendorff perfused rat heart.

Various vasoconstrictive substances, such as 5-HT, thromboxane A₂, endothelin or histamine, are believed to play an important role in the pathophysiology of variant angina (13, 19–21). It was reported that fasudil had an ability to block the effects of a wide variety of vasoconstrictive substances, such as phenylephrine, prostaglandin F₂α, 5-HT, histamine, angiotensin II, noradrenaline, dopamine, endothelin, Ca²⁺, KCl and A23187 (1, 7, 22–24), in cerebral arteries or aorta in vitro. In the present study, fasudil or hydroxy fasudil also relaxed the endothelin-, U-46619-, 5-HT- or histamine-induced contraction concentration-dependently and was similarly potent in inhibiting contraction induced by all the agonists tested in swine coronary arterial strips. Fasudil and hydroxy fasudil have the ability to block the effects of a wide variety of vasoconstrictive substances in arteries in vitro. It is thought that fasudil and hydroxy fasudil could ameliorate the severe contraction of arteries by interfering with the signal transduction pathway of the contraction of the smooth muscle cells by inhibiting the action of protein kinases, especially Rho-kinase.

The change in the ECG is closely related to the change in the oxygen supply/demand rate in ischemic heart diseases, and a number of potential mechanisms have been postulated for anti-ischemic actions including improved ischemic regional blood flow or a reduced myocardial oxygen demand. Since fasudil does not act as cardiodepressant (7), it is thought that its effects can occur independent of any reduction in myocardial oxygen consumption.

Fasudil is effective in protecting the heart against vasospasm and endothelin-induced myocardial ischemic change in conscious rabbits, and this beneficial effect can be attributed to its action of ameliorating the severe contraction of arteries. The inhibition of Rho-kinase may have implications for the development of novel therapeutic strategies for vasospastic angina patients.

REFERENCES


